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Mr. Stephen Johnson, Administrator
U.S. Environmental Protection Agency
Ariel Rios Building, 1101 -A
1200 Pennsylvania Ave., N.W.
Washington, DC 20460



PEOPLE FOR THE ETHICAL
TREATMENT OF ANIMALS

HEADQUARTERS
501 FRONT STREET
NORFOLK, VA 23510
TEL 757-622-PETA
FAX 757-622-0457

Subject: Public Comments on the HPV Challenge Program Test Plan for the Cobalt Salts of C2 and C3 Carboxylates Category by Members of the Metal Carboxylates Coalition (OM Group, Inc. and The Shepherd Chemical Company).

The following comments on the HPV Challenge Program test plan for the cobalt salts of C2 and C3 carboxylates category by members of the Metal Carboxylates Coalition (OM Group, Inc. and The Shepherd Chemical Company) are submitted on behalf of People for the Ethical Treatment of Animals, the Physicians Committee for Responsible Medicine, the Humane Society of the United States, the Doris Day Animal League, and Earth Island Institute. These health, animal protection, and environmental organizations have a combined membership of more than ten million Americans.

In our August 15, 2006 submission, we requested that EPA reopen the comment period for the metal carboxylates test plans, since, as a result of breaking up the category, the numbers of animals to be used has greatly increased and there are a number of serious scientific and animal welfare concerns that need to be addressed. This is the third set of comments that we have submitted on the new individual test plans.

The sponsoring companies are proposing to conduct an acute oral LD₅₀ test for cobalt propionate and an acute fish toxicity test for cobalt acetate. If conducted, these tests will cause the suffering and death of approximately 130 animals.

This test plan violates the following terms of the October 1999 agreement among the EPA, industry, and health, animal protection, and environmental organizations, as well as the December 2000 *Federal Register* notice reconfirming that agreement:

2. Participants shall maximize the use of existing and scientifically adequate data to minimize further testing.
3. Participants shall maximize the use of scientifically appropriate categories of related chemicals and structure activity relationships.

The sponsoring companies note that metal carboxylates readily dissociate into free metal and free acid. The proportion of dissociated salt is dependent on the pH, and the dissociation constant (pKa) is the pH at which 50% dissociation

occurs. The pKa values for the two category members as determined in studies conducted by the Metal Carboxylates Coalition are reported to be 7.58 for cobalt propionate and 7.75 cobalt acetate. These values indicate that complete dissociation will occur at the physiologically relevant pH of the mammalian stomach (pH 1.2). The sponsoring companies conclude therefore, that when administered orally, the toxicity of these metal carboxylates is due to the independent action of the respective acid and the free cobalt ion. As a result, mammalian toxicity data for the free acids and free metal ion, or its simple metal salts, can serve as surrogate data for that of the respective metal carboxylates. In support of this conclusion, the work of Stopford, et al. (2003)¹ is cited to show that cobalt chloride is similar to, or more bioavailable than, the corresponding cobalt carboxylate salts, thus making the chloride a conservative surrogate in estimating bioavailability and toxicity of the dissociated metal ion.

An acute oral LD₅₀ test is proposed for cobalt propionate, even though existing acute mammalian oral toxicity data is summarized for the other category member, cobalt acetate. The only justification offered for repeating this exceptionally agonizing test in cobalt propionate is to “further confirm” the results of the cobalt acetate study. Reducing testing by means of read-across from existing data is the rationale for establishing categories of related chemicals to begin with, and it is inappropriate not to do so in this case. Additionally, existing data is summarized for propionic acid, acetic acid and cobalt chloride – all of the category members’ dissociation products or, in the case of the cobalt ion, its simple metal salt. The theoretical discussion of metal carboxylates dissociation presented in the test plan and summarized above clearly shows that, under the conditions of the proposed test (i.e. oral administration), existing data for the dissociation products fully characterize the toxicity of the metal carboxylates in this category. The data summarized for cobalt acetate further support this conclusion by demonstrating that the LD₅₀ value for cobalt acetate, when expressed on the cobalt ion content, is very similar to that for cobalt chloride. Existing data on dissociation products have been used to meet SIDS requirements for compounds which readily dissociate at low pH in a number of test plans. In particular, this approach was endorsed by EPA and all stakeholders for E. I. du Pont de Nemours & Company’s test plan for triisopropylborate, a compound which breaks down to isopropanol and boric acid in water.

Further, the OECD guidelines which the proposed test would follow are not specified. It is crucial to note that OECD 401 has been phased out in favor of OECD 425 and that the EPA now recommends the use of *in vitro* cell toxicity tests to establish the starting dose for acute toxicity tests (<http://www.epa.gov/oppt/chemrtk/toxprtow.htm>) in order to further reduce the number of animals used when an acute mammalian test is perceived to be necessary.

A fish acute toxicity test is proposed for cobalt acetate. No reliable ecotoxicity data for aquatic plants or invertebrates exist for either member of this category. The fish test is intended to show whether exposure to these metal carboxylates will result in large-scale fish death thereby predicting economic loss and ecologic damage. If this exposure kills the food on which fish subsist, it could deplete fish populations even without direct fish toxicity. Since the toxicity of these metal carboxylates to aquatic plants and invertebrates is still unknown, a test on fish is premature. In addition, ECOSAR and non-animal ecotoxicity tests, such as the DarT test² and TETRATOX test³ should be considered. If a fish acute toxicity test is still perceived to be required, ECVAM’s Ecotoxicology Task Force recently published an evaluation of a fish acute

threshold (step-down) test concept with the potential to reduce the number of fish used in ecotoxicity testing by 53.6%-71.2%.⁴

In summary, while the sponsoring companies summarize existing data for cobalt acetate and all of the category members' dissociation products and present a convincing theoretical argument for the use of dissociation product data to serve as surrogates for those of the respective metal carboxylates, they nevertheless fail to use this analysis to minimize animal testing as instructed by the EPA in both the October 1999 letter to chemical sponsors and the December 2000 *Federal Register* notice on the HPV program. Instead, the sponsoring companies propose an acute oral LD₅₀ for cobalt propionate. We urge the sponsoring companies and the EPA to reject this proposed test as well as to consider the applicability of the suggested alternatives to the fish acute toxicity test.

Sincerely,

Joseph Manuppello
Research Associate
Research & Investigations

¹ Stopford W., Turner J, Cappellini D, and Brock T. 2003. Bioaccessibility testing of cobalt compounds. *J. Environ. Monit.* 5(4): 675-680.

² Nagel, R. 2002. DarT: the embryo test with the zebrafish *Danio rerio*: A general model in ecotoxicology and toxicology. *ALTEX* 19 (Suppl. 1), 38-48.

³ Schultz, T.W. 1997. TETRATOX *Tetrahymena pyriformis* population growth impairment endpoint: A surrogate for fish lethality. *Toxicological Methods* 7, 289-309.

⁴ Jerama, S., et al. 2005. A strategy to reduce the use of fish in acute ecotoxicity testing of new chemical substances notified in the European Union. *Regulatory Toxicology and Pharmacology* 42, 218-224.